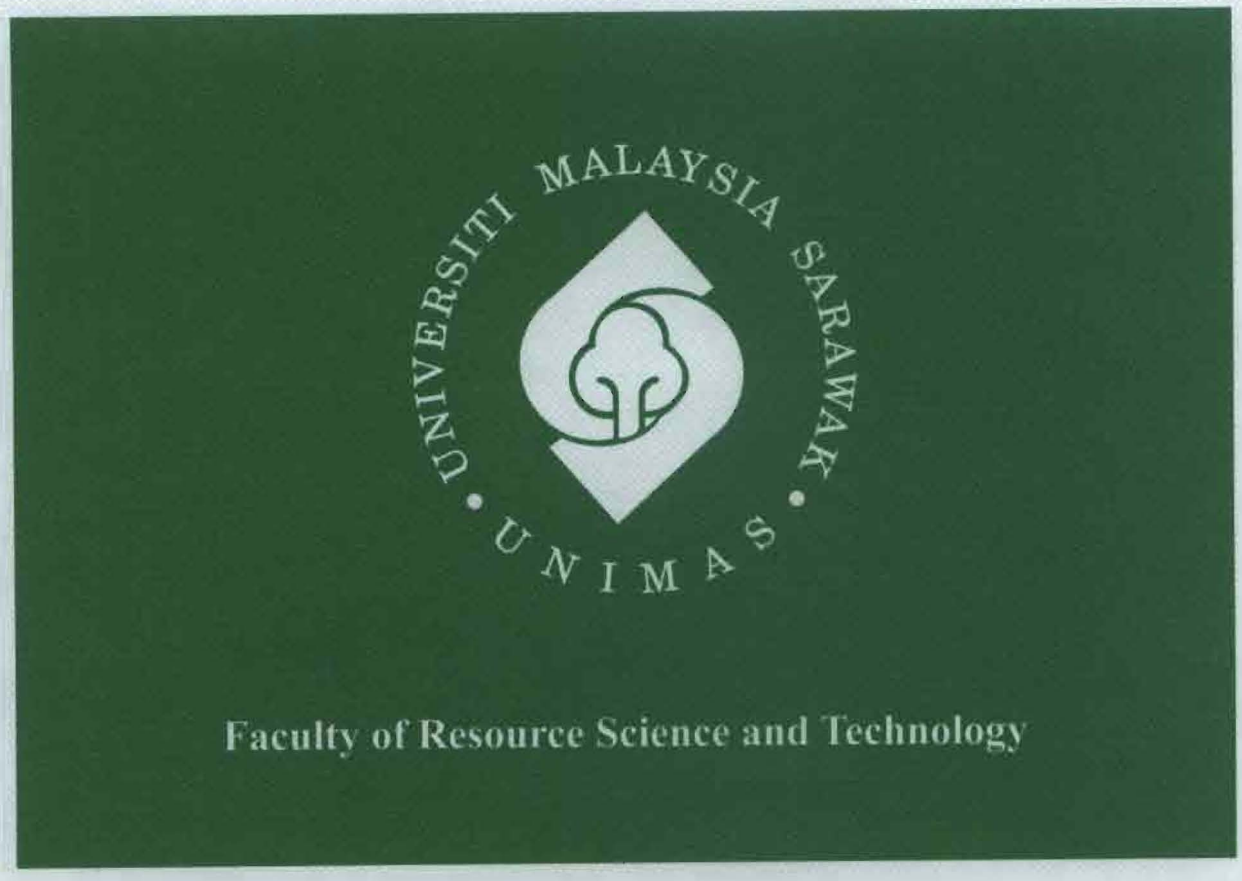

Sea star *Stellaster childreni* (Echinodermata: Asteroidea) from Exclusive Economic Zone Bintulu and Miri, Sarawak

Lee Suet Yee

Bachelor of Science with Honours
(Aquatic Resource Science and Management)
2016

Sea star *Stellaster childreni* (Echinodermata: Asteroidea) from Exclusive Economic Zone Bintulu and Miri, Sarawak

LEE SUET YEE

41803

This dissertation is submitted in partial fulfilment of the requirement for the degree of Bachelor of Science with Honours in Aquatic Resources Science and Management

Aquatic Resource Science and Management
Department of Aquatic Science

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2016

Acknowledgment

Firstly, I would like to express my deep thankful to my supervisor, Dr. Ruhana Hassan for her kind help, dedicated guidance, encouragement and support that had assisted and supervised me until the end of this final year project. Besides that, I would like to express my appreciation on her hard work and comments to improve this thesis. Next, I would like to immense my grateful towards my respective seniors, Ms. Nurhartini Kamalia Yahya, Ms. Nursyuhaida Md. Syahid, Mr. Mohd Khairulazman and Mr. Mohd Izwan Zulaini for their good cooperation and assistance throughout this project. Other than that, I would like to thank Mr Wan Zaibidi for helping me sketching the sampling map and his guidance also. Not to forget, the laboratory assistant of Department of Resources Science and Managament, Mdm. Lucy for lending her help regarding the lab work.

Other than that, I also want to thank all my friends and my labmates, Nur Madihah bt Othman, Nur Afiefah binti Ali, Nur Hafiza bt Abd Halim, Adriana Christopher Lee and Nur Athirah bt Abd Razak for their help and support.Besides that, a special thanks to Prof. Shabdin Md. Long for his kindness to help me identifying and understanding invertebrates. I also would like to thank my friend, Chong Yin Fong for helping me drawing the illustration pictures. Last but not least, a deepest thanks to my grandparents, Mr. Thong Ah Ngow and Mdm. Lam Mooi and also my mother, Mdm. Thong Ching Ching by giving me infinite support, confidence, love and caring to accomplish this final year project successfully. Thank you.

Declaration

I, Lee Suet Yee declare that the final year project report entitled:

Sea star *Stellaster childreni* (Echinodermata: Asteroidea) from Exclusive Economic Zone Bintulu and Miri, Sarawak

and the work presented in the report are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where I have made corrections based on suggestion by supervisor and examiners, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this report is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission

Signed: 

Aquatic Resource Science and Management
Department of Aquatic Science
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak (UNIMAS)

Date: 27th June 2016

Table of Contents

List of Abbreviation

µl	Microliter
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
CIA	Chloroform-isomyl alcohol
CTAB	Cetyltrimethyl Ammonium Bromide
COI	Cytochrome Oxidase I
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diaminetetra-acetic acid
EEZ	Exclusive Economic Zone
EtBr	Ethidium Bromide
EtOH	Ethanol
MEGA	Molecular Evolutionary Genetic Analysis
$MgCl_2$	Magnesium Chloride
min	Minute
ml	Millilitre
mtDNA	Mitochondria DNA
PCR	Polymerase Chain Reaction
rpm	Rotation per minutes
s	Seconds
TAE	Tris-acetate-EDTA
UV	Ultraviolet
V	Volt

List of Tables

List of Figures

Sea star *Stellaster childreni* (Echinodermata: Asteroidea) from Exclusive Economic Zone Bintulu and Miri, Sarawak

Lee Suet Yee

Aquatic Resource Science and Management
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Seastar locally known as "Tapak sulaiman" which belonging to Class Asteroidea, Phylum Echinodermata. In Malaysia, study of sea stars had been carried out in coastal water near Merambong Shoal, Johor and coral reef in the Central South China Sea. However, there is no documentation on diversity of sea stars inhabiting Sawarak EEZ. Therefore, the aim of this research is to identify sea star collected from EEZ near Bintulu and Miri, Sarawak based on morphological data, anatomy data and cytochrome oxidase I (COI) gene analysis. In addition, diet analysis, distribution and abundance of *Stellaster childreni* had been carried out. About 3.23% (n=7) specimens were found in EEZ Miri and 96.77% (n=210) specimens were found in EEZ Bintulu. However, the distribution and abundance of *S. childreni* in this study are still in underestimated because it is bycatch organism. Organisms such as gastropod juveniles, bivalve juveniles, limpets, barnacles, sponge spicule, seagrass, seaweed and benthic foraminiferans served as main food sources of *S. childreni.* About 83.67% (n=41) *S. childreni* fed on benthic foraminiferans, followed by seagrass and seaweed which about 61.22% (n=30), gastropod juvenile with 36.73% (n=18), bivalve juvenile with 12.24% (n=6), limpets, scaphopod juvenile, barnacle and sponge spicule was about 4.08% (n=2), respectively. In term of seabed types, about 41.01% (n=89) of *S. childreni* were found at mixture of sandy and muddy substratum, followed by mixture of sandy and coral 19.34% (n=42), 17.51% (n=38) of *S. childreni* were found at muddy substratum, 11.52% (n=25) were at coral substratum and 10.56% (n=23) were sandy. However, for the molecular data, negative result was obtained.

Keywords: Asteroidea, Exclusive Economic Zone, *S. childreni*, distribution

ABSTRAK

Tapak sulaiman adalah dalam Kelas Asteroidea, Filum Echinodermata. Ia dapat ditemui di kawasan-kawasan cetek dan lautan dalam. Di Malaysia, kajian mengenai tapak sulaiman telah dijalankan di air persisiran yang berhampiran Merambong Shoal, Johor dan terumbu karang di Laut China Selatan. Namun begitu, tiada kajian mengenai taburan tapak sulaiman dijalankan di EEZ Sarawak. Justeru, tujuan kajian ini adalah untuk mengenal pasti tapak sulaiman yang dikumpulkan dari EEZ berhampiran Bintulu dan Miri, Sarawak berdasarkan data morfologi, anatomi data dan cytochrome oxidase I (COI) gen analisis. Tambahan pula, analisis diet, taburan dan kelimpahan Stellaster childreni turut dikajikan. Sebanyak 3.23% (n=7) S. childreni ditemui berhampiran EEZ Miri dan 96.77% (n=210) S. childreni telah ditemui berhampiran EEZ Bintulu dari Bintulu. Namun begitu,taburan dan kelimpahan S. childreni yang diperolehi dalam kajian ini masih tidak tepati sebab ia ditangkap begitu sahaja. Organisma seperti juvenil gastropod, juvenil kerang, limpet, teritip, span spicule, rumput laut, rumpai laut dan foraminiferans bentik adalah sebagai sumber makanan utama S. childreni. Sebanyak 83.67% (n=41) S. childreni makan foraminiferans bentik, diikuti oleh rumput laut dan rumpai laut yang kira-kiranya sebanyak 61.22% (n=30), gastropod juvenil yang sebanyak 36.73% (n=18), kerang jevenil yang sebanyak 12.24% (n=6), limpets, jevenil scaphopod, teritip dan span spicule masing-masing adalah sebanyak 4.08% (n=2). Mengikut jenis dasar laut, kira-kira 41.01% (n=89) daripada S. childreni ditemui di campuran pasir dan lumpur, kemudian diikuti dengan campuran pasir dan batu karang mananya kira-kira 19.34% (n=42), 17.51% (n=38) daripada S. childreni ditemui di dasar laut lumpur, 11.52% (n=25) adalah dari dasar terumbu karang dan 10.56% (n=23) adalah dari dasar berpasir. Namun begitu, bagi data molekul, keputusan negative telah diperolehi.

Kata kunci: Asteroidea, Exclusive Economic Zone, S. childreni, taburan

1.0 Introduction

Echinodermata is an invertebrate, and found in tropical to cold seafloor. Echinodermata includes feather stars and sea lilies (Crinodea); starfish or seastars (Asteroidea); brittle and basket stars (Ophiuroidea); sea urchins (Echinoidea); and sea cucumber (Holothuroidea). Echinodermata plays major role in marine ecosystem and some species have economic value, for example sea urchin roe is an expensive and famous food in Korean and Japanese cuisine, whereas extract from sea cucumber contains collagen which is important in pharmaceutical field (Putchakarn and Sonchaeng 2004). Majority of Echinodermata members are marine species while only a few are categorised as brackish species.

Asteroid is commonly known as star fish or sea star which is a type of marine invertebrate that occurs from intertidal to abyssal deep sea area where may achieve approximately 6000 m depth (Mah and Blake 2012). Asteroid comprises approximately 1900 extant species classified into 36 families, and nearly 370 extant genera (Mah and Blake 2012). Asteroids serve as "keystone species" due to their predatory nature such as *Pisasteris* that interacts with *Mytilusand* along the coast of North American (Calil *et al.*, 2009) and are documented as determining factors in distribution pattern, abundance as well as density (Alvarado and Solis-Marin, 2013; Chamundeesari *et al.* 2013). Therefore, they usually served as model organism (Selvaraju, 2013).

Comprehensive study to describe the species and density of asteroid in Sarawak region is relatively limited. Sim *et al.* (2008) had done a research in coral reef area which located in Central South China Sea. They had discovered two new species of Asteroidea namely

Fromia sp. and *Leiaster* sp. The most recent study in Malaysia water was conducted by Woo *et al.* (2014) in seagrass bed of Merambong Shoal near Johor. They reported that a total of five species from four families were found where three of the species were the first record in Malaysian water. The three new discovered species were *Stellaster equestris*, *Luidia maculate* and *Goniodiscaster scaber*.

In both Sim *et al.* (2008) and Woo *et al.* (2014) studies, they collected samples from shallow coral reef and seagrass bed, respectively. However, in this study, a collection was done from Exclusive Economic Zone (EEZ) near Miri, Sarawak using trawler. In this study, asteroids were found on benthic substrates which is different from previous studies. Furthermore, previous studies only involved shallow water. A quick search in the National Centre for Biotechnology Information (NCBI), there are 4683 cytochrome oxidase I (COI) gene sequence data from various species of Asteroidea, and only two COI gene sequence data from *Stellaster childreni*. Both are from Tasmania coast, Australia (Ward *et al.*, 2008). There is no available molecular data from *Stellaster childreni* originated from Sarawak. Therefore, the aim of this research is to identify asteroid that collected in EEZ near Miri, Sarawak based on morphological data and sequence analysis of COI gene. Upon completion of this study, data obtained will be made available for future study.

2.0 Literature Review

2.1 Asteroidea

2.1.1 Morphology

Asteroidea is motile echinoderm with radial symmetry. In addition, Asteroidea can be characterized by a flattened body and gradually narrower into arms. Basically, asteroids have endoskeleton which flexible naturally and separated ossicles are formed. Selvaraju (2013) stated that water vascular system, haemal system and radial nervous system are developed well in asteroids. He also reported that asteroids absent definite brain, eye, heart and blood but the nervous system relatively sensitive with external stimulation.

Asteroidea usually comprises of five arms but some species have more or less and the presented arms are always in triangular shape and originated from central disc (Sim *et al.*, 2008). Based on Mah and Blake (2012), each arm possess a series of J-shaped ambulacral ossicles along the arm radius. Ambulacral grooves are emerged a series of tube feet and these grooves present on oral side. However, tentacles that present in each arm usually comprise of ocelli which function in light and dark identification (Selvaraju, 2013).

Blake (1987) had proposed the following characters during diagnosis of a species, (1) general body appearance including shape of body, general nature of skeleton and encrusting ossicles, (2) nature of major ossicular system, starting from abactinally and continue around the body and develop into interior, (3) soft parts such as papulae and tube feet, and (4) developmental characters. Selvaraju (2013) claimed that shape of pedicellariae is one of the main characteristics in asteroids taxonomy.

2.1.2 Taxonomy of asteroids

Stellaster childreni belongs to Genus Stellaster of the Family Goniasteridae. The Family Goniasteridae is under Order Valvatida. Taxonomy of *Stellaster childreni* was stated by Gray (1840):

Kingdom: Animalia

Phylum: Echinodermata

Class: Asteroidea

Order: Valvatida

Family: Goniasteridae

Genus: *Stellaster*

Species: *Stellaster childreni* (Gray, 1840)

2.1.3 Feeding behaviour

Based on Karleskint *et al.* (2010), asteroids are either carnivores or scavengers and some are bottom feeders. Asteroids *Luidia* and *Asteropecten* inhabit soft bottom habitats and thus they can feed on the preys which burry in sediment. A typical asteroids use their tube feet in feeding which located in ambulacral groove. Each arms contains digestive glands whereas the stomach and anus are located at central disc (Karleskint *et al.*, 2010).

Based on Himmelman and Dutil (1991), prey preferences vary among asteroids species. Species inhabit in the intertidal areas consume mussels as mussel beds are available

in that area. While the adult asteroids inhabit area with below 8 m in depth are likely to consume clams. They stated that the young asteroids feed on small mussels and barnacles while the adult asteroids prefer larger prey such as larger bivalves. Most of the asteroids could change their prey selection as they increase in size.

Each arm contains digestive glands. Feeding behaviour of asteroids are unique. Firstly, asteroids wrap around the prey and pry the valves apart. A portion of stomach is averted out from central mouth and inserted into the prey where they digested the prey. After finished feeding, stomach is drawn back into their mouth and it shifted away from their prey (Karleskint *et al.*, 2010).

2.1.4 Distribution (Habitat)

Based on Chamundeeswari *et al.* (2013), asteroids inhabit various ecosystems such as seagrass, coral or rocky substratum and from shallow water to deepest region in the ocean. These ecosystems have redirect impact on different feeding behaviour practised by asteroids. Dabis *et al.* (2014) suggested that the diversity of asteroids depends on by depth gradient. They reported that occurrences of the highest species diversity occurs between the surface up to 1000 m depth. Declination of species diversity and richness occur after 1000 m depth of the ocean. In contrast, Howell *et al.* (2002) reported that almost all asteroids were found only between depth ranges of 200-300 m. Other than that, they stated that factors such as food availability and interaction with environment are likely to influence the distribution of asteroids rather than the physiological parameters like temperature and pressure.

2.1.5 Reproduction of Asteroids

Reproduction mode is depending on the species of asteroids. Asteroids usually are dioecious although some species are hermaphrodites and some may change sexes when condition is unfavourable (Selvaraju, 2013).

One of the reproduction mode is sexual reproduction at which fertilization process occurs externally. Large number of larvae are produced which can regarded as zooplankton (Selvaraju, 2013). There is usually one breeding season per year and a single female asteroids may release about 2.5 million eggs. In most asteroids, the released eggs and larvae stage are planktonic although some species from Arctic and Antarctic brood their eggs under the central disc (Karleskint *et al.*, 2010).

They can also reproduce asexually by fragmentation in which separated their arms and part of the central disc and then can be develop into independent individual from parent (Selvaraju, 2013). Each half then regenerates the missing part of body organ like disc and arms although the extra arms are commonly produced (Karleskint *et al.*, 2010).

2.1.6 Importance of Asteroids

According to Mah and Blake (2012), asteroids have both ecological and pharmaceutical uses. Extraction and isolation of natural chemical substances as sterols can be obtained from asteroids (Bos *et al.*, 2008). Chamundeeswari *et al.* (2012) found that asteroids contain saponins, sterol, anthraquinones and phospholipids and are served as useful components in pharmaceutical field. The steroidal glycosides and related compounds are predominant metabolites in sea stars such as saponins related in anti-inflammatory and

immune-stimulating activity (Sahellan, 2014) while anthraquinones contains anti-cancer properties (Huang *et al.*, 2007).

Other than that, asteroids are one of the targeted species for aquarium trade (Bos *et al.*, 2008). Wood (2001 as cited in Bos *et al.*, 2008) claimed that two third of global trade is supplied from Indonesia and Philippines. There are living asteroids sold in markets and also the dried specimen, especially the juvenile of *Archaster typicus* as decoration in bathroom and souvenirs in tourist shops throughout Europe (Bos *et al.*, 2008).

Corresponding to ecological importance, asteroids serves as indicator in marine pollution and toxicological studies. Several genera have become subjects in the uptake of toxic metals, PCBs and effect of oil such as *Asterias*, *Evasterias* and *Coscinasterias* (Mah and Blake, 2012).

Moreover, asteroids diversity is generally correlated with other invertebrates in same environment and thus asteroids can as key indicator of high biodiversity hold for other taxa (Waay-Juico, 2014). Asteroids have great impact on benthic community and feeding ecology since they have main role in food chain and thus current status of biological knowledge must be well understood in order to manage the resources in a more sustainable manner.

2.2 Previous studies about Asteroids

Sim *et al.* (2008) reported that there are limited studies on the distribution and taxonomy of asteroids in Malaysian water. They found that there were six families, 12 genera and 20 species of asteroids found in Central South China Sea and that 20 species were similar to results from Purwati and Lane (2004) who identified 28 species that belong to 9 families from Anambas and Natuna Archipelagos (Southern South China Sea). There were only 8

species namely *Echinaster luzonicus*, *Fromia monilis*, *F. indica*, *Linckia laevigata*, *L. multifora*, *Choriaster granulatus*, *Culcita novaeguineae* and *Neoferdina offreti* recorded in both central South China Sea and Southern South China Sea (Purwati & Lane, 2004; Sim *et al.*, 2008).

Woo *et al.* (2014) had updated the record of asteroids in Malaysian water. They examined seagrass habitats at the southern coast of Peninsular Malaysia and found five species of asteroids from four families. Three of the species found were the first records for Malaysia namely *Stellaster equestris, Luidia maculate* and *Goniodiscaster scaber*. They also reported that these three species are widely distributed in Indo-Pacific area.

2.3 Exclusive Economic Zone (EEZ)

Based on article 55 of United Nations Convention, EEZ is an area beyond the adjacent to the territorial sea subject to the specific legal regime established in this Part, under which the rights and jurisdiction of the coastal State and the rights and freedoms of other States are governed by the relevant provisions of this Convention. EEZ only include 200 nautical miles from baseline from which the breadth of the territorial sea is measured (Division for Ocean Affairs and the Law of the Sea, United Nations, 2013).

Long term interest and essential properties should under protection eithers laws, policies or acts. Maritime Enforcement Agency is one of the responsible agency to protect these interests especially in EEZ areas (Othman, 2012). With respect to the EEZ, sovereign rights over all natural resources as well economic activities, educational research purpose and environmental protection and conservation are bond by Exclusive Economic Zone Act 1984 (Act *311)*. Malaysian government has jurisdiction with regards to manage the EEZ areas.

2.4 Cytochrome oxidase I (COI) gene

Mitochondrial deoxyribonucleic acid (mtDNA) is a circular chromosome that found in mitochondrial which about 17 000 base pairs (bp) that encoded most replication activity. MtDNA genes are widely used as markers to resolve phylogenetic relationship and infer species boundaries. DNA barcoding is a process that use a short DNA sequence from standard locus for example using COI gene (Nicolas *et al.*, 2012). COI gene is one of the mtDNA genes that could be used in species identification tool and to articulate all animal life (Nicolas *et al.*, 2012; Roe and Sperling, 2007).

Ward *et al.* (2008) reported that COI gene information had been useful in echinoderm discrimination. The DNA sequence of COI gene from asteroids were retrieved from the Genebank on 4 October 2015 which both of the samples were from Western Australia. Only two COI gene data from various species of Asteroidea were obtained (http://www.ncbi.nlm.nih.gov). Thus, in this research, COI gene are proposed to be used for DNA barcoding in determine the phylogenetic relationship of asteroids specimens.

2.5 Polymerase Chain Reaction (PCR)

Analysis of Polymerase Chain Reaction (PCR) is one of the key constituent in molecular biology to amplify a single or several copies of DNA sequence in order to generate thousand to million copies of DNA sequence. Kary Mullis, an American biochemist invented this PCR analysis in 1984 and received Nobel Prize and Japan Prize in 1993. PCR analysis now become one of the most popular method which applied widely in medical and biological lab work. This is because PCR analysis is quick, affordable pricing and simple to use (Joshi and Deshpande, 2010).

Principle behind PCR is relatively simple as only involving amplification of DNA. As its name, it is a chain reaction at which one DNA molecule is able to generate two copies, then four, then eight and so on. In this series of chain reactions, there are three major steps, denaturation, annealing and extension. In denaturation step, the double stranded DNA is denatured into two single stranded DNA at high temperature (Joshi and Deshpande, 2010). Next, in annealing, lower temperature is required in order to allow the primers anneal to DNA template and this is the most significant step as if the primers bind to correct targeted position in DNA template, then expected yield will be obtained (Palumbi, 1996). In extension, enzymatic reaction is taken place where the targeted DNA segment will be synthesized, complimentary copy strand of DNA is created according to Chargaff's rule.

3.0 Materials and methods

3.1 Samples collection

The sampling for this research was done by UNIMAS team and crews of M.V. SEAFDEC 2 on 22 August to 28 August 2015 at EEZ where near Bintulu as shown in Figure 1. There were 217 individuals of asteroids were collected using trawler. Trawling was carried out for 1 hour using beam trawl as shown in Figure 2 at pre-selected stations with different depth between 18 m to 185 m. The habitat types (substrata and depth) were recoded. Samples were kept in plastic bags, labelled and brought to the Aquatic Molecular Laboratory, FRST UNIMAS for further analyses. In the laboratory, samples were stored in -80 °C deep freezer. All samples were subjected to morphological assessment based on identification key (VandenSpiegel *et al.*, 1998) and proceeded to total genomic DNA extraction using modified CTAB (Doyle and Doyle, 1987) followed by PCR experiments and DNA sequencing.

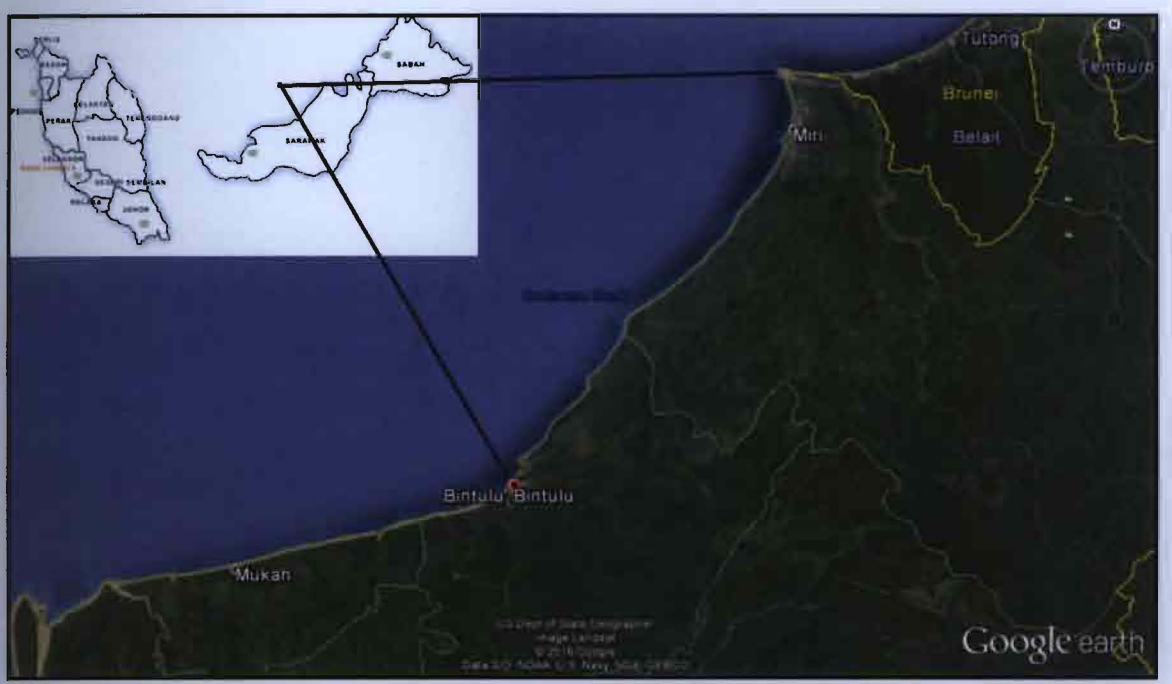

Figure 1. Sampling areas near Bintulu and Miri, Sarawak (source: Google Earth, 2016)

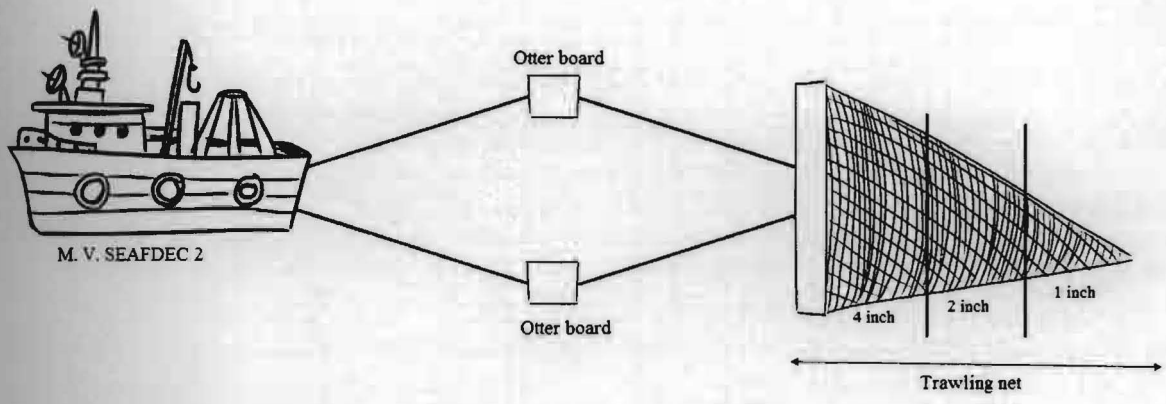

Figure 2. Illustration of trawling method practiced by M.V. SEAFDEC 2.

3.2 Morphological examination and measurement

Morphological characteristics were examined by following VandenSpiegel *et al.* (1998). Measurement based on the length of arms (R) and radius from disc to groove (r) were taken by using ruler up to 0.1 mm. Morphological investigation such as mouth, central disc, arms other characteristic such as pigmentation that present on oral surface was carried out and recorded.

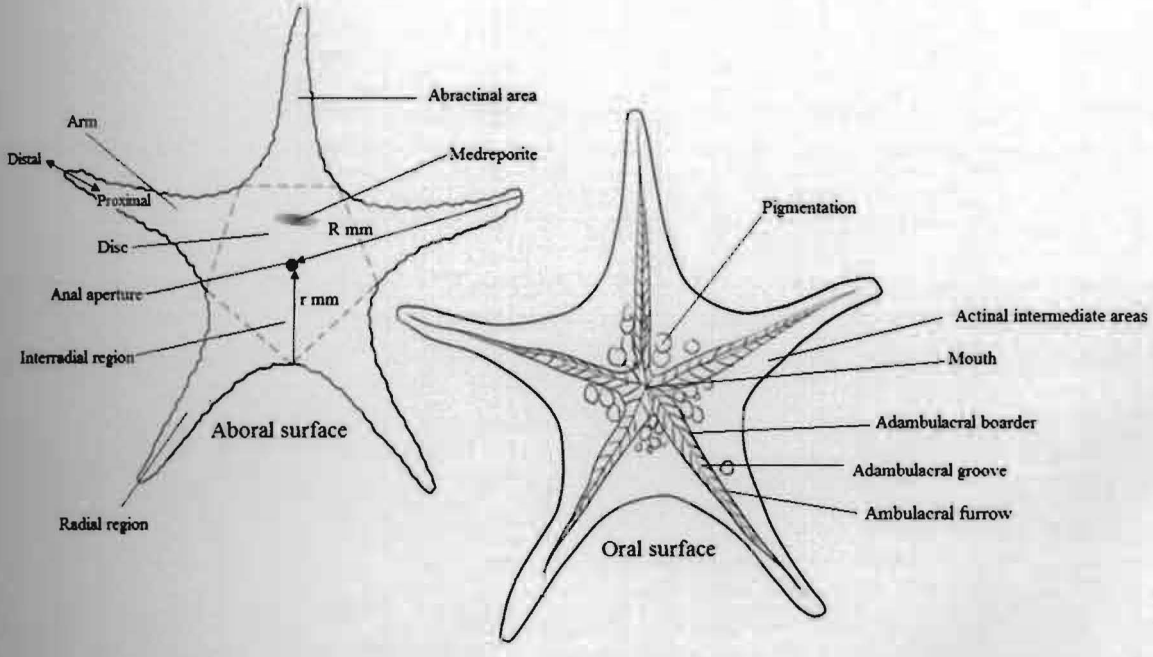

Figure 3. Explanation of terminology of the asteroid body form (adapted from VandenSpiegel *et al.*, 1998).

3.3 Feeding behaviour analysis

Stomach contents of *S. childreni* were examined (n=49). About 5 individuals were selected randomly as representative of each station. Stomach from each station was dissected out by using steriled blade. Then, the stomach contents were isolated by using forcep. Small hard structures, sediment grains, sclerites or spicules, and aquatic macrophytes were isolated and preserved in 10% formalin and labelled. The stomach contents were identified under stereo microscope (Motic Microscope) and image was captured. The feeding behaviour analysis was done by calculated prey items of each taxa using following formulae (Lima-Junior and Goitein, 2001):

$$F_i = (100\ n_i) \div N$$

Where, F_i = Frequency of occurrence of the *i* food items in *S. childreni*

n_i = Number of stomach that *i* food items is found

N = Total number of stomachs with food in the specimens

3.4 Molecular Work

3.4.1 Preparation of tissue samples for total genomic DNA extraction

Firstly, the samples were taken out from freezer carefully. The frozen samples of asteroids were thawed and washed by using distilled water to remove foreign particles. The tissue samples were cut-sliced by using surgical blades and placed properly in labelled tubes. Surgical blade was renewed every time when cut-sliced new tissue samples. The prepared samples were stored in -20 °C deep freezer for further work.